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# Inflammasomes in rheumatoid arthritis: a pilot study

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## Abstract

**Background** The inflammasome plays an important role in rheumatoid arthritis (RA), which has rarely been systematically reported. The aim of this study was to understand whether the levels of inflammasomes were related to the severity of RA disease, which might provide a stronger theoretical basis for RA treatment.

**Methods** The *mRNA* expression levels of some inflammasomes and associated molecules, including *IL-1beta* and *IL-18*, in peripheral blood mononuclear cells (PBMCs) from 30 RA patients (n = 30) and 16 healthy control (HC) individuals were determined by quantitative real-time polymerase chain reaction (qRT-PCR), and the levels of plasma IL-1beta and IL-18 were also measured by enzyme-linked immunosorbent assay (ELISA). Moreover, the clinical characteristics and laboratory results of the patients were collected and analyzed in this study.

**Results** The relative *mRNA* expression levels of *NLRP3*, *NLRC4*, *AIM2*, *caspase-1*, and *IL-1beta* were significantly higher and those of *NLRP1*, *NLRP2* and *NLRC5* were notably lower in the HC group than in the RA group. Moreover, the plasma IL-1beta and IL-18 levels were markedly increased in the RA group. Additionally, the *mRNA* level of *AIM2* was negatively correlated with disease activity score 28 (DAS28) by stepwise linear regression analysis. erythrocyte sedimentation rate (ESR) was positively correlated with DAS28 by multiple linear regression analysis in the RA group.

**Conclusions** These findings imply the critical role of *NLRP3*, *NLRC4*, *AIM2*, *caspase-1* and plasma IL-1beta and IL-18 in the pathogenesis of RA patients, which provides potential targets for the treatment of RA.

**Keywords** Inflammasome, Rheumatoid arthritis, *NLRP3*, *NLRC4*, *AIM2*

## Background

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic erosive arthritis. The pathogenesis is marked by persistent, chronic inflammation of the synovial membrane. This leads to synovitis and damage to bone and joints, which ultimately causes joint deformity, loss of function, and even disability [1, 2]. The pathogenesis of RA remains unclear, although inflammasomes are considered to be closely involved in RA pathogenesis. Inflammasomes are an important component of the immune system. They identify pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs), or homeostasis-altering molecular processes (HAMPs) on pathogens through pattern

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recognition receptors (PRRs), recognizing and eliminating exogenous pathogenic microorganisms or endogenous molecules released by the host itself [3, 4]. The canonical inflammasomes are composed of a sensor molecule, an adapter protein (apoptosis-associated speck-like protein containing a caspase recruitment domain, ASC), and an effector molecule [5]. The sensors include members of the NLR family, the absent in melanoma 2 (AIM2)-like receptor (ALR) family and pyrin, specifically NOD-like receptor protein 1 (NLRP1), NLRP2, NLRP3, NOD-like receptor family apoptosis inhibitory protein (NAIP), NLR family CARD domain-containing 4 (NLRC4), NLRC5, NLRP6, NLRP7, NLRP9, NLRP12, AIM2, human interferon (IFN)  $\gamma$ -inducible protein (IFI) 16 and pyrin [6, 7]. Caspases include human caspases-1/4/5 and mouse caspases-1/11 and active inflammatory caspases that cleave pro-IL-1 $\beta$  and pro-IL-18 to produce active IL-1 $\beta$  and IL-18. Thus, they exert proinflammatory effects and promote the cleavage of the pore-forming protein gasdermin D (GSDMD) to induce pyroptosis [5, 8].

Inflammasomes are expressed in immune cells, such as T cells, monocytes, and macrophages, and nonimmune cells, such as epithelial cells and myofibroblasts [5]. An

appropriate inflammatory response is beneficial for the body. However, it will trigger excessive tissue damage and lead to disease when it is uncontrolled. While there are numerous reports on inflammasomes in RA, no systematic studies have been reported.

In this study, we assessed the mRNA levels of *NLRP1*, *NLRP2*, *NLRP3*, *NLRC4*, *NLRC5*, *NLRP12*, *AIM2*, *CARD8*, *IFI16*, *Pyrin*, *NAIP*, *caspase-1*, *caspase-4*, *caspase-5*, *IL-1 $\beta$* , and *IL-18* in PBMCs from RA patients and HC individuals and conducted a pilot investigation of common inflammasomes and associated molecules to provide a stronger theoretical basis for RA treatment.

## Materials and methods

### Objective

This study aimed to perform a pilot study on the expression levels of inflammasomes and associated molecules to provide a stronger theoretical value for RA treatment.

### Study design and participants

A total of 30 patients with RA were enrolled in this study; these patients included 10 males and 20 females. The average age of these patients was 55 years (range: 31 to 75 years). RA patients who fulfilled the revised American College of Rheumatology (ACR) 2010 criteria for RA were consecutively enrolled in the Shaoxing People's Hospital between October 2021 and March 2022 [9]. RA patients with other inflammatory or autoimmune diseases, cancers, mental disorders or hormonal diseases and pregnant and lactating women were excluded from this study. Information on disease activity score 28 (DAS28), rheumatoid factor (RF), anti-cyclic citrullinated peptide antibodies (anti-CCP), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), white blood cell (WBC) count, neutrophil count, monocyte count, lymphocyte count and other data were collected. A total of 16 healthy control individuals (HC individuals) matched for sex and age were also enrolled. This group included 5 males and 11 females of 40 to 72 years of age who had no autoimmune or inflammatory diseases. The characteristics of these enrolled patients are summarized in Table 1.

### Human PBMC separation and total RNA extraction

Fresh peripheral blood samples were collected into EDTA-K<sub>2</sub>-containing tubes and centrifuged at 800  $\times$  g for 5 min. Then, the plasma was collected into the tubes and stored at -80  $^{\circ}$ C. The remaining blood components were diluted with an equal volume of phosphate buffered saline (PBS), and then PBMCs were collected by gradient centrifugation according to the Lympholyte<sup>®</sup>-H (CEDARLANE, Netherlands) manufacturer's protocols. The total RNA in PBMCs from each case was extracted using an RNA Extraction Kit (Vazyme Biotech,

**Table 1** Clinical details of the RA and HC groups

	RA (n = 30)	HC (n = 16)
<b>Demographics</b>		
Sex, male/female	10/20	5/11
Age (range)	55 (31–75)	57 (40–72)
BMI (kg/m <sup>2</sup> )	22.4 $\pm$ 4.1	
Smoking (Yes/No)	8/22	
Alcohol drinking (Yes/No)	10/20	
<b>Clinical characteristic</b>		
Disease duration, years (range)	6.37(0.3–16)	
DAS28-CRP	3.66 $\pm$ 1.79	
RF, IU/ml	255.20 $\pm$ 237.70	
Anti-CCP, RU/ml	366.90 $\pm$ 476.27	
ESR, mm/h	36.60 $\pm$ 27.52	
CRP, mg/l	16.91 $\pm$ 22.07	
plasma IL-1 $\beta$ , pg/ml	6.62 $\pm$ 1.65***	4.93 $\pm$ 0.98
plasma IL-18, pg/ml	759.79 $\pm$ 661.36**	307.89 $\pm$ 230.42
WBC, 10 <sup>9</sup> /l	5.96 $\pm$ 1.87	5.38 $\pm$ 1.25
N, 10 <sup>9</sup> /l	3.87 $\pm$ 1.64	2.97 $\pm$ 1.01
M, 10 <sup>9</sup> /l	0.43 $\pm$ 0.17	0.39 $\pm$ 0.09
L, 10 <sup>9</sup> /l	1.78 $\pm$ 1.16*	1.86 $\pm$ 0.37
<b>Treatments</b>		
Glucocorticoids, n (%)	12 (40%)	
Methotrexate, n (%)	18 (60%)	
Biologic agents, n (%)	8 (27%)	
JAK inhibitors, n (%)	5 (17%)	

Note: DAS28-CRP, disease activity score for 28 joints based on C-reactive protein; RF, rheumatoid factor; BMI, body mass index; anti-CCP, anti-cyclic citrullinated peptide antibody; ESR, erythrocyte sedimentation rate; IL-1 $\beta$ , interleukin-1 $\beta$ ; WBC, white blood cell count; N, neutrophil count; M, monocyte count; L, lymphocyte count. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

Nanjing, China) as quickly as possible according to the protocol. The integrity and concentration of the RNA were assessed by a NanoDrop 2000 (Thermo Scientific, USA) spectrophotometer.

#### qRT-PCR analysis

Quantitative real-time polymerase chain reaction (qRT-PCR) was carried out with the PrimeScript™ RT kit (Vazyme Biotech, Nanjing, China) and SYBR Premix Ex Taq™ II (Vazyme Biotech, Nanjing, China). The house-keeping gene encoding GAPDH was used as an internal control, and the primers are listed in Table 2. The relative expression levels of the target genes were analyzed using the  $2^{-\Delta\Delta C_t}$  method.

**Table 2** The amplification primer sequences

Gene name	GeneBank accession no.	Primer sequence (5'-3')
<i>NLRP1</i>	NM_033004	F-ATTGAGGGCAGGCAGCACAGAT R-CTCCTTCAGGTTTCTGGTGACC
<i>NLRP2</i>	NM_017852	F-CATTCTGCGTCAAGCACTGTCTG R-CCGTCCAGAAAGGAAGCATGTG
<i>NLRP3</i>	NM_004895	F-GGACTGAAGCACCTGTTGTGCA R-TCCTGAGTCTCCCAAGGCATTC
<i>NLRC4</i>	NM_021209	F-AGGTCCCACAACCTCGTCAAGCT R-TGCTCACACGATTTCCCGCAA
<i>NLRC5</i>	NM_032206	F-AGTGGCTCTCCGCTTGGACAT R-CGGAACCCTAAGAAGCTGGCTG
<i>NLRP12</i>	NM_144687	F-CAGGCATGATGCTGCTTTGCGA R-AGCACAGAAGCCATCTCTGAC
<i>AIM2</i>	NM_004833	F-GCTGCACCAAAGTCTCTCCTC R-CTGCTTGCCCTTCTGGGTCTCA
<i>CARD8</i>	NM_014959	F-GTGTGGGATACTGAGGTGAAGC R-TGTCTTGAGATCATCGAGCAC
<i>IFI16</i>	NM_005531	F-GATGCTCCATCAACACCAAGC R-CTGTTGCGTTCAGCACCATCAC
<i>Pyrin</i>	NM_000243	F-GCTGCTCTTCTGTGAGGATCAC R-CTCAGCTTCTCAGATGCTCCAG
<i>NAIP</i>	NM_004536	F-CCGAACAGGAAGCTTCTCTCAC R-CCACAGACAGTCTTTTCAGGCAC
<i>Caspase-1</i>	NM_033292	F-GCTGAGGTTGACATCACAGGCA R-TGCTGTCAGAGGCTTGTGCTC
<i>Caspase-4</i>	NM_001225	F-GGGATGAAGGAGCTACTTGAGG R-CCAAGAATGTGCTGTCAGAGGAC
<i>Caspase-5</i>	NM_004347	F-ACAACCGCAACTGCCTCAGTCT R-GAATCTGCCTCCAGGTTCTCAG
<i>IL1beta</i>	NM_000576	F-CCACAGACCTTCCAGGAGAATG R-GTGCAGTTCAGTGATCGTACAGG
<i>IL-18</i>	NM_001562	F-GATAGCCAGCCTAGAGGTATGG R-CCTTGATGTTATCAGGAGGATTCA
<i>GAPDH</i>	NM_002046	F-GTCTCCTCTGACTTCAACAGCG R-ACCACCCTGTGCTGTAGCCAA

Note: NLRP1, NOD-like receptor protein 1; AIM2, absent in melanoma 2; IFI16, human interferon (IFN)  $\gamma$ -inducible protein 16; NAIP, NOD-like receptor family apoptosis inhibitory protein

#### Enzyme-linked immunosorbent assay (ELISA)

The plasma concentrations of IL-1beta and IL-18 were determined by enzyme-linked immunosorbent assay (ELISA) according to the protocol of the kit (Boster Biological, Wuhan, China). The detection wavelength was 450 nm, and experiments were conducted in triplicate with consistent results.

#### Statistical analysis

Statistical analysis and graphic presentation were performed by SPSS version 25.0 and GraphPad Prism 9.0. Unpaired Student's t test or one-way ANOVA was used when the data passed the normality test; otherwise, the nonparametric Mann-Whitney test or the Kruskal-Wallis test was used. The correlation analysis was performed by multiple linear regression analysis and stepwise linear regression analysis, excluding the interference of confounding factors. Two-tailed  $P < 0.05$  was considered to be statistically significant.

## Results

#### Characteristics of study subjects

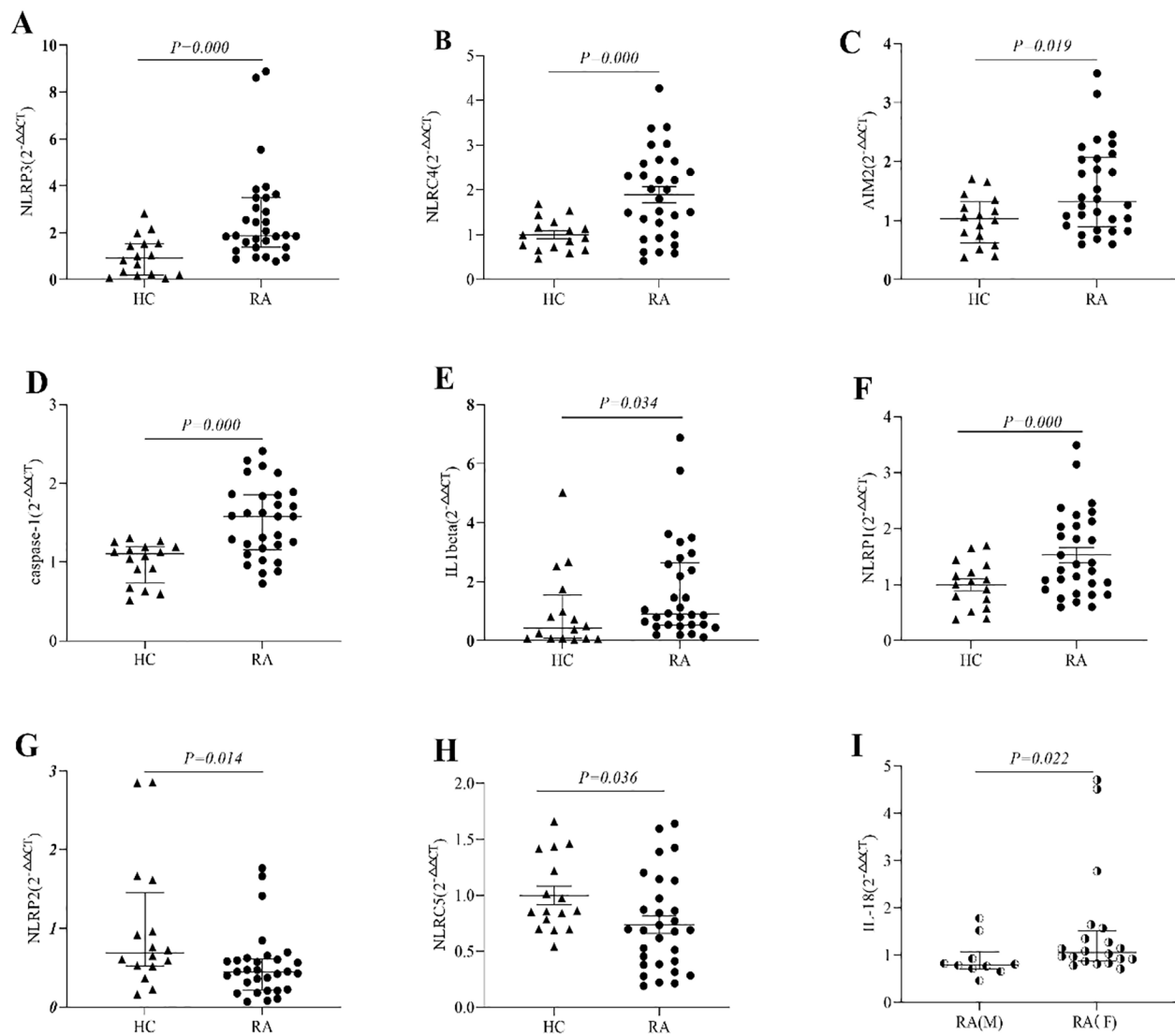
Thirty patients with RA and sixteen healthy adults were enrolled in this study. There was no sex or age differences between the RA group and the HC group. There was no significant difference in the counts of WBCs, monocytes and neutrophils between the two groups, but the number of lymphocytes in the RA group was higher than that in the HC group ( $P = 0.04$ ).

#### The mRNA expression levels in the RA and HC groups

The mRNA expression levels of *NLRP3*, *NLRC4*, *AIM2*, *caspase-1* and *IL-1beta* in the RA group were markedly increased (Fig. 1A-E), but the levels of *NLRP1*, *NLRP2* and *NLRC5* were notably decreased in comparison with those in the HC group (Fig. 1F-H). Moreover, there were no significant differences in *NLRP12*, *CARD8*, *IFI16*, *pyrin*, *NAIP*, *Caspase-4/5* and *IL-18* mRNA levels between the two groups (Supplementary material: Table S1). Although there was no significant difference in the relative expression levels of *IL-18* mRNA between the two groups, the female group was markedly higher than the male group in RA patients (Fig. 1I).

#### Inflammasomes and DAS28-CRP in RA patients

To assess whether these inflammasomes and related proteins differed at different disease activity levels, the RA group was divided into high activity ( $\text{DAS28-CRP} > 5.1$ ), moderate activity ( $3.2 < \text{DAS28-CRP} < 5.1$ ), low activity ( $2.6 < \text{DAS28-CRP} < 3.2$ ), and remission ( $\text{DAS28-CRP} < 2.6$ ) groups according to DAS28-CRP [9]. The *NLRP1* mRNA levels in the RA with different activity groups (low, moderate and high activity) were noticeably different from those in the HC group, and there



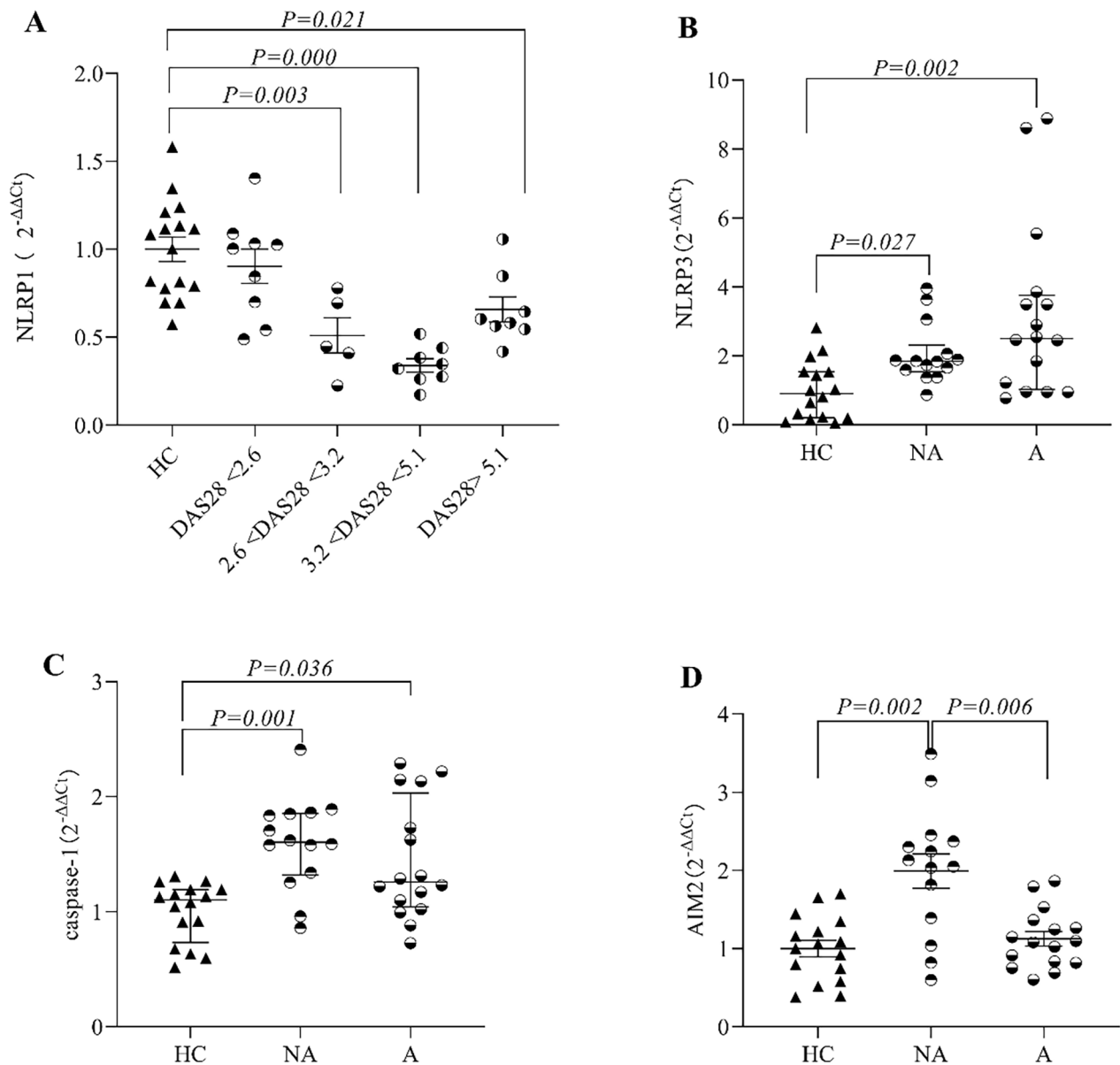
**Fig. 1** Differential expression levels of inflammasomes and associated molecules. (A) The mRNA level of *NLRP3*. (B) The mRNA level of *NLRC4*. (C) The mRNA level of *AIM2*. (D) The mRNA level of *caspase-1*. (E) The mRNA level of *IL-1beta*. (F) The mRNA level of *NLRP1*. (G) The mRNA level of *NLRP2*. (H) The mRNA level of *NLRC5*. (I) The mRNA level of *IL-18* in females with RA. RA, rheumatoid arthritis group; HC, healthy control group; M, male; F, female

were no significant differences between the RA remission group and the HC group. The relative expression levels of *NLRP1* mRNA were not markedly different among the different RA activity groups (low, moderate and high activity) (Fig. 2A). The comparison between the active (including moderate and high activity groups) and inactive (low activity and remission groups) RA groups indicated that the mRNA levels of *NLRP3* and *caspase-1* in both the inactive and active groups were higher than those in the HC group, but there were no notable differences between the inactive and active groups (Fig. 2B, C). The levels of *AIM2* mRNA in the inactive group were the highest and were significantly different from those in the HC group and the active group (Fig. 2D). The CRP and ESR levels in the high activity group were markedly

higher than those in the low activity group and the remission group.

#### Measurement of plasma IL-1beta and IL-18

To determine whether there were differences in the plasma IL-1beta and IL-18 levels between RA patients and HC groups, these levels were determined by ELISA. The plasma IL-1beta ( $6.62 \pm 1.65$ ,  $P=0.000$ ) and IL-18 ( $759.79 \pm 661.36$ ,  $P=0.001$ ) levels in the RA group were noticeably higher than those in the HC group (Fig. 3A, B). There were noticeable significant differences among the active, inactive, and HC groups (Fig. 3C), but no difference was observed between the active and inactive groups. The level of IL-18 was the highest in the active



**Fig. 2 Differences in the expression of inflammasomes in different groups.** (A) *NLRP1* mRNA levels in the DAS28-CRP > 5.1 (high activity group), 3.2 < DAS28-CRP < 5.1 (moderate activity group), and 2.6 < DAS28-CRP < 3.2 (low activity group) groups were noticeably lower than those in the HC group, but there were no significant differences between the DAS28-CRP < 2.6 (remission group) and HC groups. (B) *NLRP3* mRNA levels in the inactive and active groups were higher than those in the HC group. (C) *Caspase-1* mRNA levels in the inactive and active groups were higher than those in the HC group. (D) *AIM2* mRNA levels in the inactive group were higher than those in the HC group and the active group. IA, inactive group; A, active group

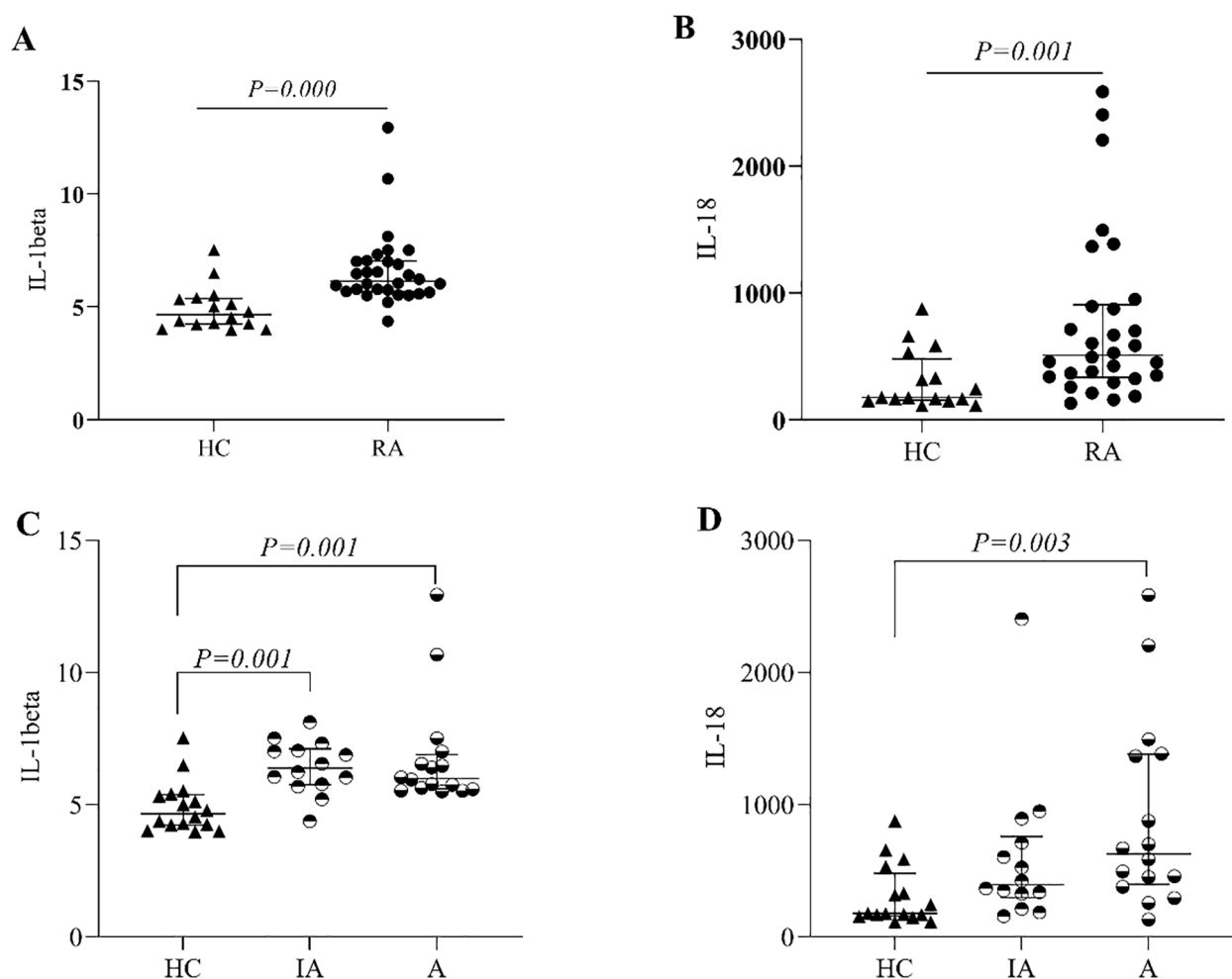
group, which was markedly different from that in the HC group (Fig. 3D).

**Correlation between mRNA expression levels and clinical features of RA patients**

To explore the relationships among the mRNA expression levels of these inflammasomes, plasma IL-1beta, IL-18 levels and clinical characteristics of RA patients, multiple linear regression analysis was used to analyze the relative mRNA expression levels of *NLRP1*, *NLRP2*,

*NLRP3*, *NLRP4*, *NLRP5*, *NLRP12*, *AIM2*, *CARD8*, *IFI16*, *Pyrin*, *NAIP*, *Caspase-1*, *Caspase-4*, *Caspase-5*, *IL-1beta*, *IL-18* mRNA and plasma IL-1beta and IL-18 levels and the clinical characteristics of RF, anti-CCP, CRP and ESR. However, no significant difference was found.

To find out whether the inflammasome mRNAs above affect plasma IL-1β and IL-18 levels, the results of stepwise regression analysis showed that the mRNA expression of inflammasome *NLRP1* (β=-0.351, P=0.017, 95% CI -3.068 to -0.323, R squared after adjustment 0.123)



**Fig. 3** Differential expression assay of plasma IL-1beta and IL-18. (A) The plasma levels of IL-1beta in the RA group were noticeable higher than those in the HC group. (B) The plasma levels of IL-18 in the RA group were markedly higher than those in the HC group. (C) The plasma levels of IL-1beta in the inactive and active groups were higher than those in the HC group. (D) The plasma levels of IL-18 in the active group were higher than those in the HC group

was negatively correlated with plasma IL-1 $\beta$  and that the *mRNA* expression of *IL-18* ( $\beta=-0.351$ ,  $P=0.017$ , 95% CI -3.068 to -0.323, R squared after adjustment 0.123) was positively correlated with plasma IL-18 levels.

To understand the correlation between the expression level of these inflammasomes and related molecules and disease activity, multiple linear regression analysis and stepwise linear regression analysis were conducted. The results showed that the *mRNA* level of *AIM2* was negatively correlated with DAS28 ( $\beta=-0.420$ ,  $P=0.021$ , 95% CI -1.832 to -0.162, R squared after adjustment 0.147). Additionally, to determine whether the laboratory findings were associated with DAS28, we compared the RE, anti-CCP, CRP, ESR, and neutrophil, monocyte and lymphocyte counts with DAS28 using multiple linear regression analysis. The results showed that ESR was positively correlated with DAS28 ( $\beta=0.711$ ,  $P=0.01$ , 95% CI 0.013 to 0.083, R squared after adjustment 0.623).

## Discussion

It is well known that the inflammasome is an important part of the innate immune system. Inflammasome activation triggers a series of inflammatory cascade responses, leading to the secretion of various cytokines, including the production of IL-1beta and IL-18. This has implications for the pathogenesis of many diseases, such as RA, systemic lupus erythematosus (SLE) and influenza virus infection [10]. In view of the role of inflammasomes in RA disease, we investigated the relative *mRNA* expression levels of common inflammasomes, including *NLRP1*, *NLRP2*, *NLRP3*, *NLRC4*, *NLRC5*, *NLRP12*, *AIM2*, *CARD8*, *IFI16*, *Pyrin* and related molecules, including *NAIP*, *caspase-1*, *caspase-4*, *Caspase-5*, *IL-1beta* and *IL-18*, in human PBMCs of RA patients and HC individuals.

The results showed that the relative *mRNA* expression levels of *NLRP3*, *NLRC4*, *AIM2*, *caspase-1*, and *IL-1beta*

in the RA group were noticeably higher than those in the HC group. However, the relative *mRNA* expression levels of *NLRP1*, *NLRP2*, and *NLRC5* were notably decreased in the RA group compared to those in the HC group. Interestingly, the *mRNA* levels of *IL-18* were significantly higher in the female group than in the male group in RA patients. Although the lymphocyte count in the RA group was higher than that in the HC group, the two groups were comparable because the present study compared relative expression levels in human PBMCs. PBMCs mainly contain lymphocytes (including T-lymphocytes, B-lymphocytes, NK cells, etc.) and monocytes. In this study, inflammasomes mainly in monocytes and T lymphocytes of RA patients were activated, causing a cascade of reactions that affected the plasma levels of IL-1beta and IL-18 [10]. Therefore, we examined the plasma levels of IL-1beta and IL-18 in the RA and HC groups and found that the plasma IL-1beta and IL-18 levels in the RA group were markedly higher than those in the HC group. To further explore whether plasma IL-1beta and IL-18 can reflect disease activity and inflammation, we correlated inflammasome *mRNA* levels and cytokines with DAS28, CRP, ESR, RF and anti-CCP. The results showed that the *mRNA* level of *AIM2* was negatively correlated with the DAS28 and that the ESR was positively correlated with the DAS28.

Wang et al. showed that *NLRP3* and *IL-1beta* *mRNA* levels in human PBMCs were not significantly different between the RA patients and HC individuals, and *caspase-1* *mRNA* levels were lower than controls. These results are different from the results of the present study, probably due to factors such as disease duration or drug use [11]. We divided *NLRP3*, *caspase-1* and *IL-1beta* *mRNA* into five or three groups according to DAS28-CRP, and there was no noticeable difference between the groups (data not shown). These findings suggest that these *mRNAs* were not highly expressed due to increased disease activity. Some studies have shown that tofacitinib (TOF) attenuates *NLRP3* activation and reduces serum IL-1beta production [12]. *NLRP3* expression is increased in adjuvant arthritis (AA) and collagen-induced arthritis (CIA) animal models [13, 14]. Both IL-1beta and *NLRP3* levels are increased in the PBMCs of RA patients [15, 16], suggesting that *NLRP3* exacerbates the inflammatory response in RA. Moreover, inhibitors targeting *NLRP3* have been applied to a variety of clinical diseases with good clinical efficacy [8]. Therefore, *NLRP3* plays a pathogenic role in the disease process of RA.

Our study showed that *AIM2* levels were significantly higher in the RA group than in the HC group, which was consistent with a previous report [17]. After we divided *AIM2* into three groups according to disease activity, the results showed a significant difference between the active, inactive and HC groups. The highest *AIM2* levels were

found in the inactive group, which might explain why the relative *mRNA* levels of *AIM2* were negatively correlated with DAS28. Animal models of *AIM2* deficiency exhibit milder inflammatory responses and pathological changes [18, 19]. Although the *AIM2* levels in RA patients were lower than those in HC individuals, fibroblast-like synovial cell (FLS) proliferation was inhibited after *AIM2* was silenced in FLSs from RA patients [20]. Although the results were inconsistent, *AIM2* remains a target for RA treatment according to the current findings. Additionally, there are fewer studies on *NLRC4* in RA, and only one study from Brazil supports the role of *NLRC4* in RA [21]. Our results showed higher *NLRC4* levels in the RA group than in the HC group. In conclusion, both *AIM2* and *NLRC4* may play pathogenic roles in the RA disease process.

Gene polymorphisms of *NLRP2* are associated with susceptibility to RA in the Chinese Han population [22]. There are no other reports on *NLRP2* in RA, but the present study first found that the *NLRP2* levels in PBMCs of RA patients were significantly lower than those in HC individuals. However, its function still needs to be further studied. In addition, we found that the *mRNA* levels of *NLRP1* were also markedly lower in RA patients than in HC individuals, which is consistent with a previous report [11]. And *NLRP1* *mRNA* level was negatively correlated with plasma IL-1beta. After the RA group was grouped by DAS28, there was no significant difference between these groups, indicating that *NLRP1* was not associated with disease activity. Different results on whether genetic polymorphisms of *NLRP1* are associated with RA have been reported, and some studies have also reported that inflammatory conditions in animal models can be attenuated by inhibiting the *NLRP1* inflammasome [23–25]. In general, the role of *NLRP1* in RA needs to be further explored with larger sample sizes that exclude the influence of other relevant factors. *NLRC5* levels significantly increased in synovial tissues of AA rats and FLSs of RA patients, and inflammatory cytokines were significantly decreased after *NLRC5* silencing [26–28].

Genetic polymorphisms of *CARD8* have been most studied for their association with RA susceptibility or anti-TNF therapy [21, 29]. Caspase-4/5 is a downstream protein of the noncanonical pathway of the inflammasome that is rarely reported in RA, and genetic polymorphisms of caspase-5 are associated with an increased risk of RA development [30]. Studies have shown that *NLRP12* negatively regulates the phosphorylation of signal transducer and activator of transcription 3 (STAT3), and the inflammatory response is exacerbated in the *NLRP12*<sup>-/-</sup> antigen-induced arthritis (AIA) mouse model [31, 32]. The roles of the inflammasomes mentioned above need to be further investigated.

The levels of plasma IL-1beta and IL-18 were significantly higher in RA patients than in HC individuals, and there was no significant difference between the groups when IL-1beta was divided into five or three groups. The use of leflunomide has been reported to decrease the serum level of IL-18 [33]. In this experiment, the study subjects were divided into a group using leflunomide, a group using hormones and a group using both drugs. The plasma level of IL-18 was analyzed between the three groups, and there was no significant difference in our study. Vasilev et al. reported that serum IL-18 levels were significantly reduced in females [34]. In this study, there was no significant difference in plasma IL-18 levels after grouping by sex, but *IL-18 mRNA* levels were notably lower in the female group than in the male group. Furthermore, *IL-18 mRNA* was correlated with plasma IL-18. The results of this study may also suggest the use of drugs that target IL-18 in the treatment of female patients with RA.

## Conclusions

Although common inflammasomes have been gradually reported in RA, there are no systematic studies reporting multiple inflammasomes simultaneously. Our study is a more comprehensive report of common inflammasomes and their associated proteins in RA. Future studies with larger sample sizes in newly diagnosed patients with RA or with a single drug alone are needed due to sample size limitations and drug use. The human body is complex. The pathogenesis of diseases is not controlled by a single cytokine and involves complex interactions between proinflammatory and anti-inflammatory factors. For example, caspase-1 is a downstream protein of many inflammasomes, so its level can be increased or decreased by a variety of factors. Because of this, inhibitors targeting caspase-1 have also become the focus of research. For example, VX-740 (pralnacasan) attenuates osteoarthritis injury, and VX-765 (belnacasan) inhibits cytokine secretion in RA models [35, 36]. Additional inhibitors include MCC950, which directly targets the NACHT domain of the NLRP3 inflammasome, tranilast (TR) [37, 38], anakinra, which is an antagonist against the IL-1 receptor (IL-1Ra), and canakinumab, which is an anti-IL-1 $\beta$  monoclonal antibody [39, 40]. The latest study shows that *NLRP3* gene polymorphisms elevated the susceptibility to RA disease, and have an impact on RF as well as anti-CCP titers in RA patients, and pyroptosis will also be a possible therapeutic target for RA [41, 42]. With the deepening of the research on the mechanisms of the inflammasome, inhibitors of the inflammasome itself or its related upstream and downstream proteins are gradually being applied.

RA is caused by a variety of factors, genetic or environmental, and its pathogenesis and therapeutic approaches

are constantly evolving. Given the important role of inflammasomes in the disease process of RA, a deeper understanding of the mechanisms of action of each inflammasome is important for the development of RA treatment strategies. Overall, our findings indicate the important role of inflammasomes and associated molecules in the pathogenesis of RA patients and could contribute to the therapeutic strategy of RA.

## Abbreviations

PBMCs	Peripheral blood mononuclear cells
ELISA	Enzyme-Linked Immunosorbent Assay
PAMPs	Pathogen-associated molecular patterns
DAMPs	Damage-associated molecular patterns
HAMPs	Homeostasis-altering molecular processes
PRRs	Pattern recognition receptors
NLRP3	NOD-like receptor protein 3
NLRC4	NLR family CARD domain-containing 4
AIM2	Absent in melanoma 2
IFI16	Human interferon (IFN) $\gamma$ -inducible protein 16
NAIP	NOD-like receptor family apoptosis inhibitory protein
GSDMD	Pore-forming protein gasdermin D
CRP	C-reactive protein
ESR	Erythrocyte sedimentation rate
RF	Rheumatoid factor
anti-CCP	Anti-cyclic citrullinated peptide antibodies
DAS28	Disease activity score 28

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s41927-023-00353-8>.

Supplementary Material 1

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## Authors' contributions

DC, GY and RL conceived and designed the experiments. QJ, XW, XX, LH and GZ performed the experiments. QJ, XX and DC analyzed the data. QJ and XW wrote the manuscript. DC revised the manuscript, contributed reagents, materials. QJ and XW contributed equally to this work. All authors contributed to the article and approved the submitted version.

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## Data Availability

All datasets generated for this study are included in the manuscript and the Supplementary material.

## Declarations

### Ethics approval and consent to participate

This project was approved by the Ethics Committee of the Shaoxing People's Hospital (no. 2022-Y346-01) and all study protocols complied with the Declaration of Helsinki. All involved participants provided written informed consent.



**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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**References**

- Aletaha D, Smolen JS. Diagnosis and management of rheumatoid arthritis: a review. *JAMA*. 2018;320(13):1360–72. <https://doi.org/10.1001/jama.2018.13103>
- Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *Lancet (London England)*. 2016;388(10055):2023–38. [https://doi.org/10.1016/S0140-6736\(16\)30173-8](https://doi.org/10.1016/S0140-6736(16)30173-8)
- Strowig T, Henao-Mejia J, Elinav E, Flavell R. Inflammasomes in health and disease. *Nature*. 2012;481(7381):278–86. <https://doi.org/10.1038/nature10759>
- Kanneganti TD, Lamkanfi M, Núñez G. Intracellular NOD-like receptors in host defense and disease. *Immunity*. 2007;27(4):549–59. <https://doi.org/10.1016/j.immuni.2007.10.002>
- Xue Y, Enosi Tuipulotu D, Tan WH, Kay C, Man SM. Emerging activators and regulators of Inflammasomes and pyroptosis. *Trends Immunol*. 2019;40(11):1035–52. <https://doi.org/10.1016/j.it.2019.09.005>
- Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-1 $\beta$ . *Mol Cell*. 2002;10(2):417–26. [https://doi.org/10.1016/S1097-2765\(02\)00599-3](https://doi.org/10.1016/S1097-2765(02)00599-3)
- Chen L, Cao SQ, Lin ZM, He SJ, Zuo JP. NOD-like receptors in autoimmune diseases. *Acta Pharmacol Sin*. 2021;42(11):1742–56. <https://doi.org/10.1038/s41401-020-00603-2>
- Jiang Q, Wang X, Huang E, Wang Q, Wen C, Yang G, et al. Inflammasome and its therapeutic targeting in rheumatoid arthritis. *Front Immunol*. 2021;12:816839. <https://doi.org/10.3389/fimmu.2021.816839>
- Aletaha D, Neogi T, Sillman AJ, Funovits J, Felson DT, Bingham CO 3, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against Rheumatism collaborative initiative. *Arthritis Rheum*. 2010;62(9):2569–81. <https://doi.org/10.1002/art.27584>
- Shin JI, Lee KH, Joo YH, Lee JM, Jeon J, Jung HJ, et al. Inflammasomes and autoimmune and rheumatic diseases: a comprehensive review. *J Autoimmun*. 2019;103:102299. <https://doi.org/10.1016/j.jaut.2019.06.010>
- Wang T, Zhu CL, Wang S, Mo LW, Yang GD, Hu J, et al. Role of NLRP3 and NLRP1 inflammasomes signaling pathways in pathogenesis of rheumatoid arthritis. *Asian Pac J Trop Med*. 2014;7(10):827–31. [https://doi.org/10.1016/S1995-7645\(14\)60145-0](https://doi.org/10.1016/S1995-7645(14)60145-0)
- Yang X, Zhan N, Jin Y, Ling H, Xiao C, Xie Z, et al. Tofacitinib restores the balance of  $\gamma\delta$ Treg/ $\gamma\delta$ T17 cells in rheumatoid arthritis by inhibiting the NLRP3-inflammasome. *Theranostics*. 2021;11(3):1446–57. <https://doi.org/10.7150/thno.47860>
- Guo C, Fu R, Wang S, Huang Y, Li X, Zhou M, et al. NLRP3 inflammasome activation contributes to the pathogenesis of rheumatoid arthritis. *Clin Exp Immunol*. 2018;194(2):231–43. <https://doi.org/10.1111/cei.13167>
- Li XF, Shen WW, Sun YY, Li WX, Sun ZH, Liu YH, et al. MicroRNA-20a negatively regulates expression of NLRP3-inflammasome by targeting TXNIP in adjuvant-induced arthritis fibroblast-like synoviocytes. *Joint Bone Spine*. 2016;83(6):695–700. <https://doi.org/10.1016/j.jbspin.2015.10.007>
- Ruscitti P, Cipriani P, Di Benedetto P, Liakouli V, Berardicurti O, Carubbi F, et al. Monocytes from patients with rheumatoid arthritis and type 2 diabetes mellitus display an increased production of interleukin (IL)-1 $\beta$  via the nucleotide-binding domain and leucine-rich repeat containing family pyrin 3 (NLRP3)-inflammasome activation: a possible implication for therapeutic decision in these patients. *Clin Exp Immunol*. 2015;182(1):35–44. <https://doi.org/10.1111/cei.12667>
- Choulaki C, Papadaki G, Repa A, Kampouraki E, Kambas K, Ritis K, et al. Enhanced activity of NLRP3 inflammasome in peripheral blood cells of patients with active rheumatoid arthritis. *Arthritis Res Therapy*. 2015;17(1):257. <https://doi.org/10.1186/s13075-015-0775-2>
- Afroz S, Giddaluru J, Vishwakarma S, Naz S, Khan AA, Khan N. A Comprehensive Gene expression Meta-analysis identifies Novel Immune Signatures in Rheumatoid Arthritis Patients. *Front Immunol*. 2017;8:74. <https://doi.org/10.3389/fimmu.2017.00074>
- Baum R, Sharma S, Carpenter S, Li QZ, Busto P, Fitzgerald KA et al. Cutting edge: AIM2 and endosomal TLRs differentially regulate arthritis and autoantibody production in DNase II-deficient mice. *Journal of immunology (Baltimore, Md. 1950)*. (2015)194(3):873–7. <https://doi.org/10.4049/jimmunol.1402573>
- Jakobs C, Perner S, Hornung V. AIM2 drives joint inflammation in a Self-DNA triggered Model of Chronic Polyarthritis. *PLoS ONE*. 2015;10(6):e0131702. <https://doi.org/10.1371/journal.pone.0131702>
- Chen Y, Fujuan Q, Chen E, Yu B, Zuo F, Yuan Y, et al. Expression of AIM2 in rheumatoid arthritis and its role on Fibroblast-Like Synoviocytes. *Mediat Inflamm*. 2020;2020:1693730. <https://doi.org/10.1155/2020/1693730>
- Addobbati C, da Cruz HLA, Adelino JE, Melo Tavares Ramos AL, Fragoso TS, Domingues A, et al. Polymorphisms and expression of inflammasome genes are associated with the development and severity of rheumatoid arthritis in brazilian patients. *Inflamm Research: Official J Eur Histamine Res Soc [et al]*. 2018;67(3):255–64. <https://doi.org/10.1007/s00011-017-1119-2>
- Yang XL, Hu ZD, Wu Q, Liu X, Liu QJ, Zhang YC, et al. Association of polymorphisms in SPARC and NLRP2 genes with rheumatoid arthritis in a chinese Han population. *Mod Rheumatol*. 2015;25(1):67–71. <https://doi.org/10.3109/14397595.2014.903595>
- Zhang L, Dong Y, Zou F, Wu M, Fan C, Ding Y. 11 $\beta$ -Hydroxysteroid dehydrogenase 1 inhibition attenuates collagen-induced arthritis. *Int Immunopharmacol*. 2013;17(3):489–94. <https://doi.org/10.1016/j.intimp.2013.07.015>
- Goh LL, Yong MY, See WQ, Chee EYW, Lim PQ, Koh ET, et al. NLRP1, PTPN22 and PAD14 gene polymorphisms and rheumatoid arthritis in ACPA-positive singaporean chinese. *Rheumatol Int*. 2017;37(8):1295–302. <https://doi.org/10.1007/s00296-017-3762-x>
- Sui J, Li H, Fang Y, Liu Y, Li M, Zhong B, et al. NLRP1 gene polymorphism influences gene transcription and is a risk factor for rheumatoid arthritis in han chinese. *Arthritis Rheum*. 2012;64(3):647–54. <https://doi.org/10.1002/art.33370>
- Liu YR, Yang L, Xu QQ, Lu XY, Ma TT, Huang C, et al. Long noncoding RNA MEG3 regulates rheumatoid arthritis by targeting NLRCS. *J Cell Physiol*. 2019;234(8):14270–84. <https://doi.org/10.1002/jcp.28126>
- Yu H, Ding C, Dai S, Sun J, Wang S, Zhang Z. Long noncoding RNA FER1L4 regulates rheumatoid arthritis via targeting NLRCS. *Clin Exp Rheumatol*. 2020;38(4):713–23.
- Ji YR, Chen Y, Chen YN, Qiu GL, Wen JG, Zheng Y, et al. Dexmedetomidine inhibits the invasion, migration, and inflammation of rheumatoid arthritis fibroblast-like synoviocytes by reducing the expression of NLRCS. *Int Immunopharmacol*. 2020;82:106374. <https://doi.org/10.1016/j.intimp.2020.106374>
- Sode J, Vogel U, Bank S, Andersen PS, Hetland ML, Lochter H, et al. Genetic variations in pattern recognition receptor loci are Associated with Anti-TNF response in patients with rheumatoid arthritis. *PLoS ONE*. 2015;10(10):e0139781. <https://doi.org/10.1371/journal.pone.0139781>
- Rui H, Yan T, Hu Z, Liu R, Wang L. The association between caspase-5 gene polymorphisms and rheumatoid arthritis in a Chinese population. *Gene*. (2018)642:307–12. <https://doi.org/10.1016/j.gene.2017.11.032>
- Prado DS, Veras FP, Ferreira RG, Damasceno LEA, Melo PH, Zamboni DS, et al. NLRP12 controls arthritis severity by acting as a checkpoint inhibitor of Th17 cell differentiation. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*. 2020;34(8):10907–19. <https://doi.org/10.1096/fj.202000795R>
- Zhang X, Nan H, Guo J, Liu J. NLRP12 reduces proliferation and inflammation of rheumatoid arthritis fibroblast-like synoviocytes by regulating the NF- $\kappa$ B and MAPK pathways. *Eur Cytokine Netw*. 2021;32(2):15–22. <https://doi.org/10.1684/ecr.2021.0465>
- Gualberto Cardoso PR, Diniz Lopes Marques C, de Melo Vilar K, Dantas AT, Branco Pinto Duarte AL, Pitta IDR et al. Interleukin-18 in Brazilian Rheumatoid Arthritis Patients: Can Leflunomide Reduce It? *Autoimmune diseases*. (2021)2021:6672987. <https://doi.org/10.1155/2021/6672987>
- Vasilev G, Manolova I, Ivanova M, Stanilov I, Miteva L, Stanilova S. The role of IL-18 in addition to Th17 cytokines in rheumatoid arthritis development and treatment in women. *Sci Rep*. 2021;11(1):15391. <https://doi.org/10.1038/s41598-021-94841-x>
- Wannamaker W, Davies R, Namchuk M, Pollard J, Ford P, Ku G, et al. (S)-1-(S)-2-[[1-(4-amino-3-chloro-phenyl)-methanoyl]-amino]-3,3-dimethyl-butanoyl]-pyrrolidine-2-carboxylic acid

- ((2R,3S)-2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (VX-765), an orally available selective interleukin (IL)-converting enzyme/caspase-1 inhibitor, exhibits potent anti-inflammatory activities by inhibiting the release of IL-1beta and IL-18. *J Pharmacol Exp Ther.* 2007;321(2):509–16. <https://doi.org/10.1124/jpet.106.111344>
36. Rudolphi K, Gerwin N, Verzijl N, van der Kraan P, van den Berg W, Pralncasan, an inhibitor of interleukin-1beta converting enzyme, reduces joint damage in two murine models of osteoarthritis. *Osteoarthr Cartil.* 2003;11(10):738–46. [https://doi.org/10.1016/s1063-4584\(03\)00153-5](https://doi.org/10.1016/s1063-4584(03)00153-5)
37. Coll RC, Hill JR, Day CJ, Zamoshnikova A, Boucher D, Massey NL, et al. MCC950 directly targets the NLRP3 ATP-hydrolysis motif for inflammasome inhibition. *Nat Chem Biol.* 2019;15(6):556–9. <https://doi.org/10.1038/s41589-019-0277-7>
38. Huang Y, Jiang H, Chen Y, Wang X, Yang Y, Tao J, et al. Tranilast directly targets NLRP3 to treat inflammasome-driven diseases. *EMBO Mol Med.* 2018;10(4). <https://doi.org/10.15252/emmm.201708689>
39. Calabrese LH. Anakinra treatment of patients with rheumatoid arthritis. *The Annals of Pharmacotherapy.* 2002;36(7–8):1204–9. <https://doi.org/10.1345/aph.1A396>
40. Alten R, Gomez-Reino J, Durez P, Beaulieu A, Sebba A, Krammer G, et al. Efficacy and safety of the human anti-IL-1 $\beta$  monoclonal antibody canakinumab in rheumatoid arthritis: results of a 12-week, phase II, dose-finding study. *BMC Musculoskelet Disord.* 2011;12:153. <https://doi.org/10.1186/1471-2474-12-153>
41. Li RN, Ou TT, Lin CH, Lin YZ, Fang TJ, Chen YJ, et al. NLRP3 Gene Polymorphisms in Rheumatoid Arthritis and Primary Sjogren's Syndrome Patients. *Diagnostics (Basel Switzerland).* 2023;13(2). <https://doi.org/10.3390/diagnostics13020206>
42. Wu D, Li Y, Xu R. Can pyroptosis be a new target in rheumatoid arthritis treatment? *Front Immunol.* 2023;14:1155606. <https://doi.org/10.3389/fimmu.2023.1155606>

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